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Analysis of chiral drugs in environmental matrices: Current knowledge and trends in environmental, biodegradation and forensic fields

Ana Rita L. Ribeiro [a], Alexandra S. Maia [a], Cláudia Ribeiro [b, c], Maria Elizabeth Tiritan [b, c, d, *]

[a] Laboratory of Separation and Reaction Engineering-Laboratory of Catalysis and Materials (LSRE-LCM), Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal
[b] CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Rua Central de Gandra, 1317, 4585-116 Gandra PRD, Portugal
[c] Interdisciplinary Center of Marine and Environmental Research (CIIMAR), University of Porto, Edificio do Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4050-208 Matosinhos, Portugal
[d] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal

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ABSTRACT

The challenge to develop enantioselective analytical methods to quantify residues of chiral drugs (CDs) in environmental matrices is an actual and imperative issue. It is well known that enantiomers may differ in their biological activities; nevertheless, most environmental analytical methods still ignore the stereochemistry and the discrimination of the enantiomers. The knowledge about their occurrence and ecological impact in biota is crucial for an accurate risk assessment. This critical review highlights the importance of analyzing CDs in environmental matrices for various applications, emphasizing methodological trends. Chromatographic methodologies are the most used and include liquid (LC), gas (GC), supercritical fluid (SFC) and capillary electrophoresis. LC is still the most widely used though trends for greener approaches and the development of GC and SFC methods are also future directions. Some of the most recent applications, namely for environmental monitoring of surface and wastewater, biodegradation studies and forensic analysis, are presented and critically discussed.

1. Introduction

Nowadays there is a great concern about an enormous number of drugs that occur in the environment, namely pharmaceuticals and personal care products, illicit drugs, pesticides, among others [1–6]. Drugs and/or their metabolites that result from the excretion process of individuals go directly into domestic sewage. Consequently, wastewater treatment plants (WWTPs) represent a critical contribution for the wide range of drugs that can be found in the environment since they are not designed with the aim of completely eliminate small molecules at low concentrations [2]. In this sense, the non-degradable or partially removed compounds that escape biological treatment represent the bulk of effluent load in surface waters. Therefore, it is necessary to consider both aqueous (i.e., influent and effluent WWTP samples) and solid phases (i.e., sewage sludge and suspended solids) for a comprehensive understanding of the removal of each drug in WWTPs and in receiving waters, as well for their fate, distribution and effects in these compartments [2]. In the aquatic environment, the same mechanisms of elimination existing in WWTPs can occur, namely adsorption, biodegradation, hydrolysis and photodegradation [3]. Consequently, the residues of these organic compounds, their metabolites resulting from microbial degradation and other by-products generated by abiotic processes are prone to pseudo-

Abbreviations: CD, chiral drug; CE, capillary electrophoresis; CSP, chiral stationary phase; DAD, diode array detector; EF, enantiomeric fraction; GC, gas chromatography; GC–MS, GC coupled to mass spectrometry; HBCD, 1,2,5,6,9,10-hexabromocyclododecane; LC, liquid chromatography; LC–MS/MS, LC–tandem mass spectrometry; OCP, organochlorine pesticide; QTOF, quadrupole time-of-flight; SFC, supercritical fluid chromatography; TBECH, 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane; TQD, triple quadrupole detector; UHPLC, ultra-high performance LC; UV, ultraviolet; WBE, wastewater-based epidemiology; WWTP, wastewater treatment plant.

* Corresponding author. CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Rua Central de Gandra, 1317, 4585-116 Gandra PRD, Portugal.
E-mail addresses: elizabeth.tiritan@iucs.cespu.pt, beth@ff.up.pt (M.E. Tiritan).

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persist in the aquatic environment and other environmental compartments (e.g., soils and air). The problematic is also critical when reclaimed wastewater is used for irrigation [3–5]. Regarding sewage sludge or animal manure applied to agriculture soils, drugs can desorb and undergo direct leaching to groundwater aquifers with consequent contamination of potable water, plants and food chain [6].

Chiral drugs (CDs) are three-dimensional molecules with asymmetry in their structure. They follow the same path of the achiral drugs, but the environmental fate, distribution and effects can be enantioselective, leading to additional challenges on their assessment (Fig. 1) [7,8]. The most important parameter to understand the environmental life cycle of CDs is the enantiomeric fraction (EF), by analyzing its variations in the different compartments and relating it to the possible processes involved in the transfer between compartments, chemical and biological transformations [7,9]. Enantiomers may exhibit different biological effects, including toxicological properties, resulting from their enantioselective interaction with other naturally occurring chiral molecules. The biodegradation is also an enantioselective process, suggested as the most important elimination process in WWTPs [10]. The selective microbial degradation of enantiomers has been observed in both field applications and laboratory microcosms, as recently reviewed by Maia et al. [11]. Therefore, when released into the environment, CDs can suffer enantioselective degradation that imply variation on the EF values [11].

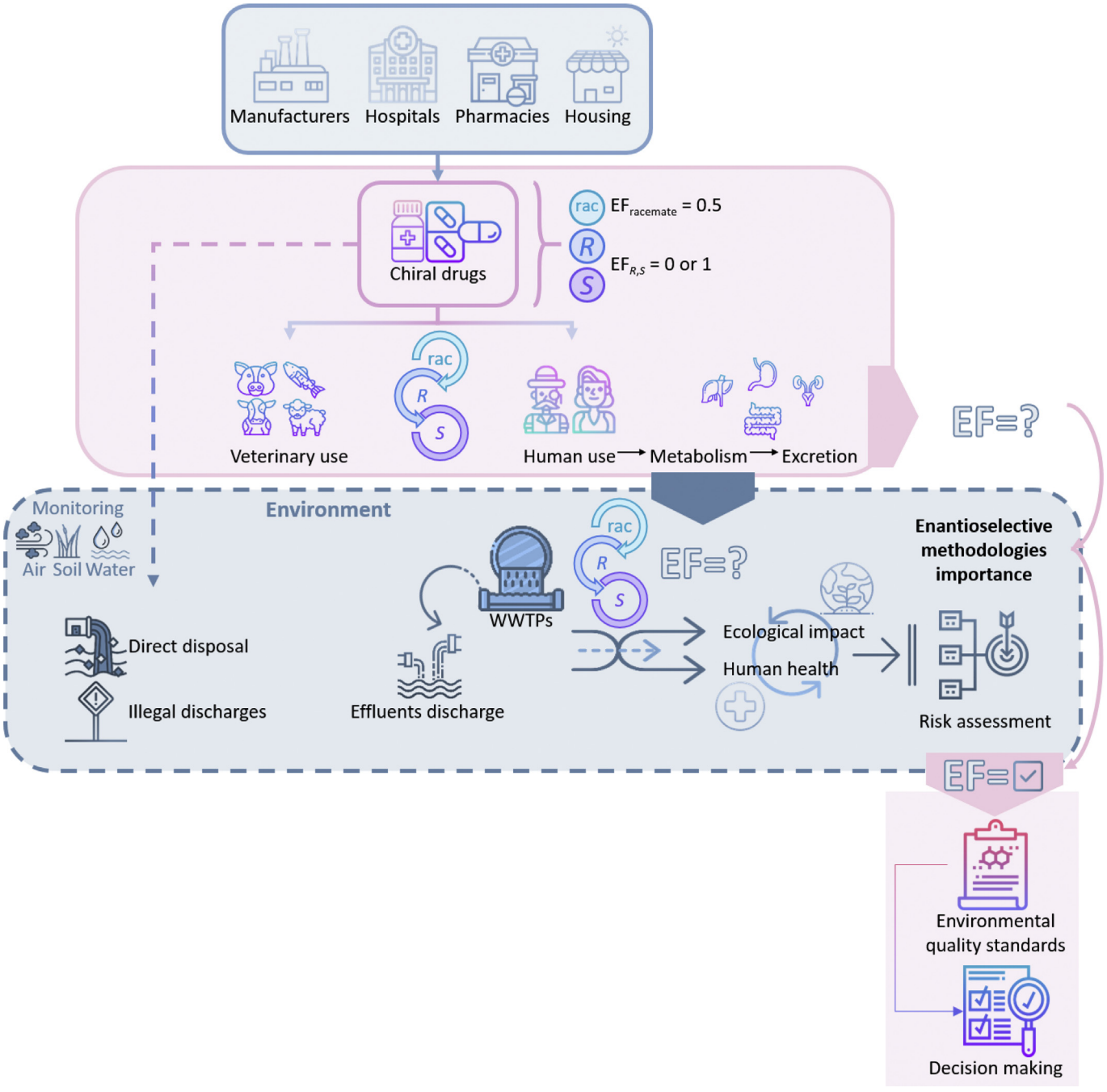

Fig. 1. The occurrence, fate and distribution framework and risk assessment of CDs in the environment.

Most studies dealing with CDs had considered enantiomers as unique molecular entities, which might lead to inaccurate data [12]. Since enantiomers may differ in their environmental behaviour (e.g. occurrence, fate, distribution, degradation, uptake) and toxicological effects, enantioselective analytical approaches are rising in interest for scientific community and many efforts are being invested in the development of new methodologies [13–16]. Despite the current understanding of the impact of stereoselectivity in the environment is still largely unknown, several reports have been published on this topic in the last decade and some reviews have already addressed this environmental concern, corroborating that enantioselective analysis is imperative [7,12,17,18]. Additionally, the knowledge of the EF in environmental samples attends not only the purpose of understanding the environmental behaviour and toxicological effects of CDs, but it is also a valuable epidemiological and forensic tool to: (i) identify sources of contamination (e.g. illegal use of pesticides or clandestine laboratories); (ii) detect illicit sewage discharges; (iii) define the consumption patterns of pharmaceuticals or illicit CDs (wastewater-based epidemiology, WBE); and (iv) identify the synthesis pathway of CDs [19]. This review aims to collect and discuss the chromatographic trends on analysis of CDs in environmental matrices for many applications, namely: environmental monitoring, biodegradation studies and forensic analysis.

2. Trends of analyses of chiral drugs in environmental matrices

The need for a better knowledge about the environmental occurrence of CDs, their fate, distribution, (bio)degradation, ecotoxicity and even the profiling for forensic purposes led to an increased interest about chiral analysis in the environment in the last decade [7,11,17–19]. In fact, information about drug contamination, their metabolites and degradation products in environmental matrices including biota, is of high importance for the determination of environmental quality and for human and animal health risk assessment. These will allow future prioritization, establishment of environmental quality standards and environmental policies, and development of remediation technologies, improving the treatment efficiency and reducing exposure and risk for both the environment and humans [20]. Even though the development of enantioselective analytical methods is still far from the progress achieved for achiral compounds, the current trends seem to be similar to those, i.e. focused on the development of greener and routine methods. The goal is to reduce and eliminate the use and generation of hazardous substances, to achieve short analysis time, to develop simple sample preparation procedures with high efficiency on the extraction of analytes, to minimize the sample amount, and to replace harmful solvents by alternative solvents such as bio-based solvents, ionic liquids or supercritical fluids [21–23]. In this sense, automation, miniaturization and reduction of waste should also be important targets in the development of new analytical methods [24].

Along with the development of novel and simple sample preparation procedures, the techniques for quantification of drug residues in environmental matrices have also been improved, allowing more reliable and sensitive methods in order to overcome the matrix complexity and the low concentration levels of the target compounds. Liquid chromatography (LC), gas chromatography (GC), and supercritical fluid chromatography (SFC) are the main analytical techniques used nowadays (Fig. 2). LC–tandem mass spectrometry (LC–MS/MS) has become the technique of choice in environmental analysis due to its versatility, high selectivity, sensitivity and the low quantification limits achieved [12,15]. Among LC–MS/MS techniques, triple quadrupole (TQD) mass analyzers remain the most used for trace analysis [8,15,25]. Even though TQD mass analyzers have been frequently used in environmental multi-residue studies, its identification potential does not necessarily represent high gains regarding the quantification ability when dealing with complex matrices and non-target compounds. Nonetheless, exact mass analyzers stand out in environmental analysis by allowing unequivocal confirmation of target compounds and structural elucidation of known and unknown metabolites/transformation products [26,27]. Hybrid mass analyzers have been gaining increased acceptance in the analysis of environmental samples due to general improvements on sensitivity, selectivity, and to the great amount of data for identification and structural elucidation purposes [28]. Particularly, the quadrupole time-of-flight (QTOF) offers high scanning speeds and a unique resolution and accuracy in mass measurements, allowing an unequivocal confirmation of target compounds and structural elucidation of unknowns [26,29]. Actually, only analytical methodologies using mass spectrometry (MS) for confirmatory purposes of the compound's identity are recognized by European guidelines as unambiguous techniques in monitoring studies (European Commission Decision 2002/657/EC) [30].

Due to the numerous chiral stationary phases (CSPs) commercially available and the high versatility provided by the multimode elution (normal, reversed, polar organic or polar ionic elution modes), LC has been the most applied technique to quantify enantiomers and to determine the EF [16,21]. The most used CSPs in environmental analyses are protein [8] and macrocyclic antibiotics-based [15,25]. Recently, Pirkle type CSPs [15] and the polysaccharide derivatives [31,32] have also been reported [33]. The recent development of CSPs with sub-2 micron particle able to be used in ultra-high performance LC (UHPLC) enable efficiency gain, increased sensitivity and peak capacity per unit time, thus allowing shorter runs and higher resolution than the conventional CSPs [34]. However, the usage of CSPs with sub-2-micron particle in environmental analysis has not been reported yet.

Interestingly, GC coupled to mass spectrometry (GC–MS) has recently resurged as a valuable technique for the environmental analysis of CDs by indirect method using chiral derivatizing reagents [14]. In fact, despite the increased use of LC–MS/MS [33], GC–MS remains as one of the most used analytical technique for analysis of chiral pesticides and volatile organic pollutants in environmental matrices [7]. Pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons or other volatile organic compounds are also analyzed in various environmental samples (water, soil and air) by GC using CSPs [7].

The enantioselective SFC methods are increasing due to the potential benefits of SFC, namely the use of CO_2 as the main mobile phase, allowing higher flow rates and thus faster separations and greener methods, since the consumption of organic solvents and the waste generation in SFC is quite lower than in LC [23]. Moreover, CO_2 can be evaporated when the pressure is reduced, recycled and purified for reuse on preparative scale, thus reducing operational costs [35]. SFC is a versatile alternative analytical methodology that allows the separation of thermolabile and non-volatile compounds that cannot be analyzed by GC and most CSPs used in LC can be also applied in SFC [23,36].

Capillary electrophoresis (CE) has been used in chiral analyses for biomedical and clinical applications [37], but its use in environmental matrices is limited [38,39]. Actually, most chiral selectors used in CSPs for LC can also be used in CE [40]. Nevertheless, other factors influencing enantioseparation, which may represent drawbacks in the development of enantioselective methods by CE, must be taken into account, namely the pH, the chiral selector concentration, the organic modifier used, among others.

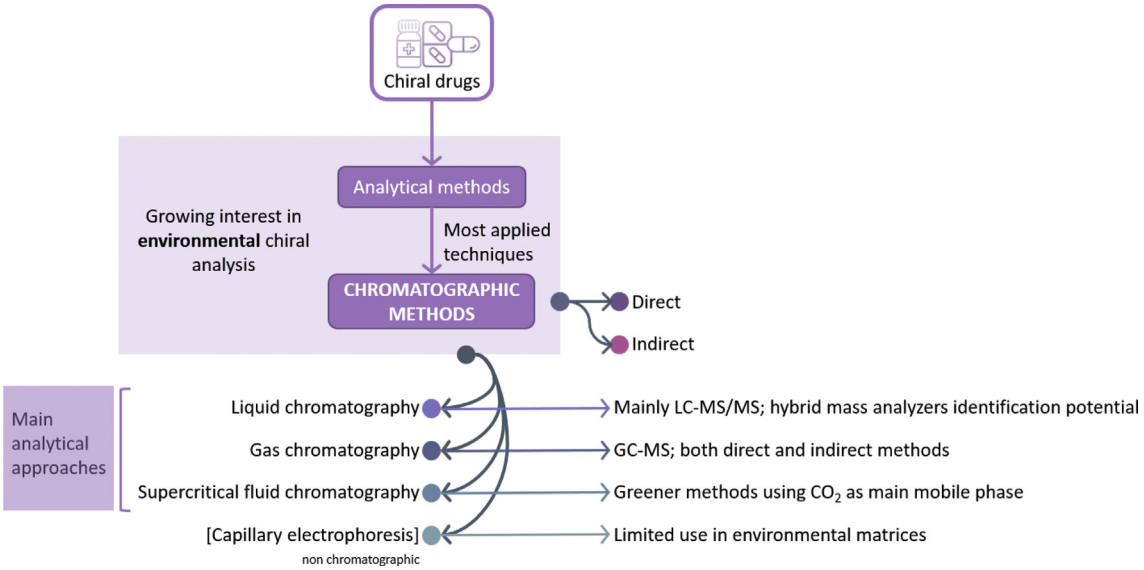

Fig. 2. The main analytical approaches of chiral analysis in environmental matrices.

Many enantioselective methods have been published [15,25,27]; however, most of them are focused on a narrow set of compounds or few classes, being multi-residue approaches still scarce. The majority of the available methodologies to quantify CDs has been applied to enantiomeric profiling in environmental monitoring, but currently the research interest of environmental chemists is also driven by: (i) their enantioselective (bio)degradation during wastewater treatment; (ii) their ecotoxicological effects; and (iii) their enantiomeric profiling in epidemiological and forensic studies.

2.1. Enantiomeric profiling in environmental monitoring

In the last decade, several enantioselective environmental monitoring programs have been published (Table 1), namely for quantification of some classes of pharmaceuticals in wastewaters and/or surface waters and pesticides in soils [7,17,21]. As recent examples, an enantioselective monitoring program using LC–MS/MS to determine three non-steroidal anti-inflammatory CDs in Chinese receiving surface waters in the Beijing area, reported the enantiomeric excess of the (*S*)-(+)-forms of ibuprofen and naproxen [41]. Petrie et al. [42] found no diurnal variability in the enantiomeric profile of several CDs, including pharmaceuticals, one metabolite, and methylenedioxymethamphetamine. Another study reported for the first time an enantioselective method suitable to determine several classes of CDs, namely beta-blockers, beta-agonists, anti-depressants, anti-histamines, and stimulants in soil samples by LC–MS/MS, showing enantioselective patterns in the degradation of some CDs [16]. A report on a two-year monitoring campaign investigated sewage and sludge samples from diverse treatment processes in four WWTPs in Hong Kong and four CDs from different pharmaceutical classes were determined [43]. The results showed much lower carrying amounts of these CDs in sludge than in the discharged effluents. Biodegradation was the primary removal mechanism of atenolol and chloramphenicol and samples from secondary treated effluents displayed enantioselectivity towards *R*-(+)-atenolol, *S*-(−)-metoprolol, and *R*-(−)-venlafaxine [43]. Interestingly, the enantiopure pharmaceutical sold *R,R*-chloramphenicol corresponded to 80% of chloramphenicol in the influents, the remaining fraction of other enantiomers being ascribed to microbial stereoisomerization before the inlets of the WWTPs or due to stereoisomeric impurities in the chloramphenicol formulations [43]. These studies on enantiomeric profiling are only some examples that demonstrate the importance of stereoselective environmental monitoring.

Another topic of environmental monitoring concern is the farming practices that could affect the persistence and enantioselectivity of CDs [31], namely wastewater irrigation and application of sewage sludge. For instance, a preferential dissipation of *R*-benalaxyl was observed in soil irrigated with tap water, wastewater and treated wastewater, with a shorter half-time of this fungicide in tap water [31]. In that study, the metabolite benalaxyl acid was enantioselectively produced, with *R*-enantiomer being predominant in the first period (21 days) and the enrichment of the *S*-form occurring afterwards. In the same report, sewage sludge accelerated benalaxyl degradation with a preferential enantioselectivity opposite of that occurring in soil, but the benalaxyl acid formed in sewage sludge was more persistent than in irrigated soil. Along with the problematic of wastewater irrigation and use of sewage sludge, the direct application of pesticides should also be monitored by enantioselective methods. The triazolinone herbicide carfentrazone-ethyl and its metabolite carfentrazone enantiomers were simultaneously determined by LC–MS/MS in agricultural and environmental samples [44]. Although carfentrazone-ethyl degradation showed no enantioselectivity, carfentrazone EF reached 0.85 after 7 days of spraying over rice plants. This study clearly reported the enantioselective natural degradation of the herbicide metabolite after its application following a standard spraying onto different plots of a rice field. An original strategy of an achiral stationary phase and a CSP in tandem was reported [52] for the separation of both the enantiomers and diastereoisomers of 4-hydroxypraziquantel, the metabolite of a broad-spectrum anthelmintic CD, praziquantel. This study monitored the depletion of *trans*- and *cis*-4-hydroxypraziquantel enantiomers in perch, tilapia, and rice field-eel muscle after oral administration and showed species-specific differences in the metabolism of the isomers [52].

Although we are facing an era of miniaturization and automation in sample preparation, these trends are still low-key approaches for chiral environmental analysis, considering the demanding extent of preconcentration when dealing with environmental matrices in general. For example, the use of online SPE as an automated sample preparation technique is still underused. A

Table 1
Summary of the different enantioseparation methods used for environmental analysis.

Compound	Matrix	Sample preparation	Analytical methodology	Chromatographic conditions	LOD	LOQ	Reference
Carfentrazone-ethyl, and carfentrazone	Cereals and soil	QueChers	LC–MS/MS	Super-chiral S-AD-RH column (150 mm × 4.6 mm i.d., 5 μm); 0.1% formic acid in a 10 mM ammonium acetate aqueous solution/methanol	0.7–6 μg kg^{-1}	2.5–20 μg kg^{-1}	[44]
Prothioconazole and prothioconazole-desthio (metabolite)	Soil	–	LC–MS/MS (triple TOF)	Lux-cellulose-1 column (150 mm × 2 mm i.d., 2 μm); acetonitrile/water (45:55, v/v)	0.0002 –0.008 mg kg^{-1}	0.005 –0.025 mg kg^{-1}	[45]
Indoxacarb	Zebrafish embryos	Acute toxicity test	LC (detector not specified)	Chiralpak IC (250 mm × 4.6 mm i.d.); dichloromethane and ethyl acetate (9:1, v/v)	–	–	[46]
Benalaxyl and benalaxyl acid	Soil and sewage sludge	Liquid–liquid extraction	LC–MS/MS	Chiralpak IC (250 mm × 4.6 mm i.d.); acetonitrile/water/formic acid (90:10:0.1, v/v/v)	Soil: 0.0015 –0.0018 μg g^{-1}; sewage sludge: 0.0031 –0.0056 μg g^{-1}	–	[31]
IBP, NAP and FBP	River	SPE (HLB)	LC–MS/MS	Chiralpak AD-RH (150 × 4.6 mm i.d., 5 μm) amylose tris(3,5-dimethylphenylcarmabate); 10 mM ammonium acetate (pH 5 adjusted with formic acid)/ acetonitrile (65:35, v/v)	0.35 –11.1 ng L^{-1}	1.1–37.1 ng L^{-1}	[41]
ATE, MET, PHO, MTZ, CIT, DCIT, FLX and MDMA	WWTP effluents	SPE (HLB)	LC–MS/MS (triple quadrupole)	Chirobiotic V (100 × 2 mm i.d.; 5 μm); 4 mM ammonium acetate in methanol containing 0.005% formic acid; Cellobiohydrolase column (100 × 2 mm i.d., 5 μm); 1 mM ammonium acetate in water/methanol (85:15, v/v)	–	–	[42]
SBT, PHO, ATE, AMP, MDMA, chlorpheniramine maleate and FLX	Soil	Accelerated solvent extraction	LC–MS/MS	Chirobiotic V2® (250 × 2.1 mm i.d., 5 μm); methanol containing 1 mM ammonium acetate and 0.01% acetic acid	0.08 –0.3 ng mL^{-1}	0.25 –1.0 ng mL^{-1}	[16]
HBCD, TBECH	Marine Food Web sediments		LC–MS/MS (for HBCD) and GC–MS (for TBECH)	LC–MS/MS: Eclipse Plus C18 column (100 × 4.6 mm i.d. mm, 3.5 μm) coupled to a NUCLEODEX β-PM column (200 × 4 mm i.d., 5 μm); GC–MS: CHIRALDEX B-TA capillary column (30 m × 0.25 mm i.d., 0.12 μm film thickness)	HBCD in sediments: 0.05 –0.2 ng g^{-1}; HBCD in biota: 0.3–0.8 ng g^{-1}; TBECH in sediments: 0.1 –0.5 ng g^{-1}; TBECH in biota: 0.8–1.8 ng g^{-1}	–	[47]
ATE, MET, VFX, CHP	WWTP influent, effluent and sludge		LC–MS (QTRAP)	Chiralpak α1-acid glycoprotein (AGP) column (150 × 3 mm i.d., 5 μm) with an AGP guard column (10 × 3 mm i.d., 5 μm)	Influent: 1.5 –7.5 ng L^{-1}; effluent: 0.3 –1.5 ng L^{-1}; sludge: 0.2 –1.0 ng g^{-1}	Table A7	[43]
IBP	Zebra fish	Metabolomic study using zebra fish adults	HPLC–UV	CHIRALCEL® OJ-H column (250 × 4.6 mm, 5 μm); *n*-hexane/2-propanol (98:2, v/v)	–	–	[48]
MFX	Blood, serum, urine	–	Differential pulse voltammetry	Cyclodextrin nanocavities: chiral selector β-cyclodextrin and graphene nanosheets (GNS)	–	–	[49]
Napropamide and acetochlor	Semi-preparative HPLC for toxicity tests (*Microcystis aeruginosa*)	Stress oxidative, Dissipation and accumulation study	LC–DAD	Napropamide: chiral OJ-H column (250 × 4.6 mm i.d., 5 μm); *n*-hexane/isopropanol (85:15, v/v); Acetochlor: chiral AS-H column (250 × 4.6 mm i.d., 5 μm); *n*-hexane/isopropanol (90:10, v/v)	0.06 –0.18 mg L^{-1}	0.07 –0.21 mg L^{-1}	[50]
Benalaxyl	Tobacco and soil	QuEChERS	USFC–MS/MS	Trefoil CEL1 (cellulose-tris-(3,5-dimethylphenylcarbamate); CO_2 and ethanol (97:3, v/v)	Soil: 0.43 –0.45 μg kg^{-1}; tobacco: 0.68 –0.72 μg kg^{-1}	Soil: 1.31 –2.01 μg kg^{-1}; tobacco 1.25 –2.15 μg kg^{-1}	[35]

(*continued on next page*)

Table 1 (*continued*)

Compound	Matrix	Sample preparation	Analytical methodology	Chromatographic conditions	LOD	LOQ	Reference
Cypermethrim	Bullfrog liver, kidney and blood	Liquid extraction, derivatization with HFIP	GC–ECD	Chiral column of BGB-17	–	0.005 mg kg^{-1}	[51]
4-Hydroxypraziquantel and metabolites	Aquaculture fish		LC–MS/MS (triple quadrupole)	Hydroxypropyl-β-cyclodextrin superficially porous particle (CDShell-RSP) column (100 × 4.6 mm i.d., 2.7 μm); 22% (v/v) acetonitrile in 10 mM ammonium formate buffer solution (pH 3.6)	–	1 μg kg^{-1}	[52]
Triadimenol, tebuconazole, fenamiphos, metalaxyl, epoxiconazole, hexaconazole, napropamide, isocarbophos, diniconazole, paclobutrazol, imazalil, fenbuconazole, propiconazole, difenoconazole, profenofos, dinotefuran, fipronil	River water, WWTP influent and effluent, soil and river sediment	Water samples: magnetic SPE; soils and river sediments: liquid extraction/ magnetic SPE	LC–MS/MS	Chiralpak IG (250 mm × 4.6 mm, i.d. 5 μm) with a guard column (10 mm × 4 mm, i.d. 5 μm); acetonitrile and ultrapure water containing 5 mM ammonium acetate and 0.05% formic acid (53:47, v/v)	Liquid samples: 0.11 –0.58 ng L^{-1}; solid samples: 0.02 –0.17 ng g^{-1}	Liquid samples: 0.35 –2.04 ng L^{-1}; solid samples: 0.08 –0.50 ng g^{-1}	[21]

AMP: amphetamine; ATE: atenolol; CIT: citalopram; CHP: chloramphenicol; DCIT: desmethylcitalopram; ECD: electron capture detector; FBP: flurbiprofen; FLX: fluoxetine; GC–MS: gas chromatography coupled to mass spectrometry; HBCD: 1,2,5,6,9,10-hexabromocyclododecane; HFIP: 1,1,1,3,3,3-Hexafluoroisopropanol; HLB: hydrophilic–lipophilic balance; IBP: ibuprofen; i.d.: internal diameter; LC–MS: liquid chromatography coupled to mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; MDMA: 3,4-methylenedioxymethamphetamine; MET: metoprolol; MFX: Moxifloxacin; MTZ: mitrazipine; NAP: naproxen; PHO: propranolol; QuEChERS: Quick, Easy, Cheap, Effective, Rugged, and Safe procedure; SBT: salbutamol; SPE: solid phase extraction; TBECH:1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane; USFC–MS/MS: ultra-high performance supercritical fluid chromatography–tandem mass spectrometry; VFX: venlafaxine.

green ultra-high performance SFC method with tandem MS using CO_2 and ethanol as mobile phase was developed by Yang et al. [35] to determine benalaxyl enantiomers in tobacco and soil, after sample preparation by the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique. An innovative approach of analysis was recently published [15], by using one single SPE cartridge to extract 23 CDs (pharmaceuticals, stimulants, and illicit drugs) in one single extraction process, without cartridge conditioning and using ethanol as solvent in the elution steps, minimizing the environmental impact by reducing waste of plastic consumables and using a green solvent. In that work, two chiral columns were used in reverse elution mode, a Chirobiotic V™ and a Pirkle type Whelk-O1®, for basic and acidic compounds, respectively. Enantioselective electrochemical sensors are another emerging field for detection of optically active molecules [49]. For example, an electrochemical chiral sensor for moxifloxacin enantiomers was developed, by fabrication of a modified carbon paste electrode using graphene nanosheets and β-cyclodextrin as chiral selector, providing unique properties namely high electrochemical response due to graphene and high supramolecular recognition due to the cyclodextrin [49]. However, to the best of our knowledge, this type of electrochemical chiral sensors was not yet applied to environmental matrices.

In the field of ecotoxicology, reports on the effects of enantiomerically pure forms on non-target organisms at environmental levels are scarce maybe due to the lack of analytical methods to support their feasibility. This type of studies are highly important, and recent examples reported the enantioselectivity in thyroid endocrine-disrupting effects due to enantiospecific binding affinities with cellular receptors for nine chiral pesticides [53], the increased oxidative responses in *Chlorella pyrenoidosa* by metconazole [54], and the stereoselective induced apoptosis in zebrafish embryos (*Danio rerio*) exposed to indoxacarb, with enantioselective tissue biodistribution [46].

For untargeted metabolic analysis, a hybrid mass spectrometer (QTOF) was required in a study concerning stereoselective effects of ibuprofen in adult zebrafish [48]. The enantioselective metabolic effects on the brain tissue of zebrafish exposed to each enantiomer and to the racemate during 28 days were investigated. In that study, 45 potential biomarkers were assessed with untargeted metabolomics, and stereoselective changes were observed for all of them. Twenty-two amino acids and three antioxidant enzymes were determined and quantified using targeted metabolomics and enzymatic assays, respectively. However, the stereochemistry of the amino acids was not specified.

Few works about enantioselective accumulation have also studied this effect at higher trophic levels, using GC as analytical method. Water exposure of bullfrog to α-cypermethrin was studied [51] by using GC equipped with an electron capture detector and a chiral column of BGB-172, based on the highly permeable skin of this amphibian. In that work, the enantioselective degradation was observed with an enrichment of (−)-(1*S*,cis,α*R*) α-cypermethrin. The metabolites cis-3-(2′,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid and 3-phenoxybenzoic acid were also detected in bullfrog tissues. Both α-cypermethrin and its metabolites accumulated mostly in liver and kidneys [51].

Trophic transfer of the chiral brominated flame-retardants 1,2,5,6,9,10-hexabromocyclododecane (HBCD, analyzed by LC–MS/MS) and 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane (TBECH, analyzed by GC–MS) was studied for the determination of the stereoisomers in sediments and thirty marine species [47]. Bioaccumulation was found to be affected by the lipid content and

biomagnification was observed, with the first eluting enantiomer of δ-TBECH being enantioselectively bioaccumulated in organisms at higher trophic levels [47].

Owing to the different physiochemical and biochemical properties that enantiomers can display, their uptake into organisms can result in the activation of defensive mechanisms and consequent metabolic responses, as reported in a study dealing with the hydrophobic herbicides napropamide and acetochlor [50]. The concentration of the enantiomers were determined by LC with photo diode array detector (DAD) and enantioseparation was achieved by using a Chiralpak® OJ-H (250 × 4.6 mm i.d., 5 μm) for napropamide and a Chiralpak® AS-H column (250 × 4.6 mm i.d., 5 μm) for acetochlor [50]. These compounds were found to diffuse into algal cells, where *S*-forms of both herbicides were dissipated faster than the *R*-enantiomers, having at the same time higher toxicity and inducing a quite superior production of antioxidant defense enzymes and microcystins, determined by commercial kits [50].

More efforts should be addressed in order to understand the unintended possible effects of both CDs and their chiral metabolites on target and non-target species for a suitable evaluation of the ecotoxicological risks associated with racemates and single enantiomers. Besides, data about concentration of CDs, their metabolites and degradation products in biota are scarce and require the development of accurate analytical methodologies. Moreover, information about trophic transfer of CDs is largely unknown despite these data being highly important for an accurate risk assessment.

2.2. Biodegradation studies

Biodegradation is considered the most important process to remove organic pollutants in conventional WWTPs and it can be enantioselective. The number of studies on the enantiomeric assessment in biodegradation studies of CDs have been increasing [11,55]. In fact, it is the most evident form to understand the stereoselectivity of biodegradation, specifically for a correct understanding of the elimination rate of each enantiomer, the eventual enrichment of one of them and the enantioselective degradation routes. Despite the different existing approaches to describe the relative abundance of enantiomers, EF is the parameter most commonly applied to assess the enantioselectivity in biodegradation studies of CDs [9]. Similarly to environmental monitoring studies of CDs, LC is the most used methodology to follow enantioselective biodegradation [11]. Regarding sample preparation, biodegradation samples tend to be cleaner than complex environmental matrices, allowing for easier sample preparation techniques that are feasible for EF quantification (Table 2). Additionally, such samples usually exhibit higher concentrations than those found in real samples and these are probably the main reasons why LC coupled to conventional detectors (UV, DAD and fluorescence) are still considered useful detection methods [27], though MS is a tendency of choice [55,56].

Despite the evolution observed in the last years in the development of enantioselective methods and biodegradation assembling experiments, studies assessing enantioselective processes linked to the formation and degradation of chiral transformation products of CDs are still limited [27,61]. The potential environmental consequences of metabolites and by-products of CDs need to be investigated regarding their occurrence, environmental fate, ecotoxicity, and their intrinsic enantioselectivity. Understanding the impact of stereoselectivity on the transformation of CDs in environmental compartments and in non-target organisms will unquestionably improve the environmental risk assessment of such compounds and give insights for future environmental regulations.

A recent example regarding the stereoselective biodegradation of several CDs was reported at both microcosm (receiving waters and activated sludge microcosms) and macrocosm (WWTP) levels using a TQD analyser and protein (cellobiohydrolase) and antibiotic-based CSPs for the enantioseparations [57]. In that study, the authors observed that some CDs marketed as racemates (e.g. fluoxetine, mirtazapine, salbutamol, methylenedioxymethamphetamine) were biodegraded with enrichment of one enantiomer in wastewater and receiving waters, sometimes the opposite enantiomer of that produced by humans. Both activated sludge and receiving waters simulating microcosms showed preferential degradation of *S*-(+)-enantiomers of amphetamines, *R*-(+)-enantiomers of beta-blockers and *S*-(+)-enantiomers of antidepressants [57]. It was observed that desmethyl metabolites of some CDs (venlafaxine, citalopram and methylenedioxymethamphetamine) had much higher removal rates both stereoselectively (metabolism) and nonstereoselectively (physicochemical processes). Besides, the metabolites were generally enriched with enantiomers of an opposite configuration to their parents, showing the importance of EF evaluation when monitoring the metabolic residues of CDs for a correct toxicological analysis (Fig. 3) [57].

In a report studying river simulating microcosms and the exposure of three different organisms (*Daphnia magna*, *Pseudokirchneriella subcapitata*, and *Tetrahymena thermophila*), the degradation and toxicity of each of the four isomers of ephedrine, enantioseparated in a CBH CSP, was followed by a LC–MS/MS (TQD) enantioselective method [58]. Similarly to other stereoselective degradation studies published [27,61], the authors described diverse degradation extents for the different enantiomers, with only two out of four being significantly degraded. Additionally, as reported elsewhere for different CDs, namely certain fluoroquinolone antibiotics [61] and antifungal pharmaceuticals [56], chiral conversion of at least one of the single enantiomers resulted from microbial metabolic processes. Moreover, the formation of 1*S*,2*R*-(+)-ephedrine in microcosms containing other enantiomers was observed, which was ascribed to microbial metabolic processes instead of being human-derived, and confirmed by MS-based data mining and human liver microsome assays [58]. Environmental racemization is becoming a frequently debated subject in recent chiral environmental studies and has motivated the development of enantioselective monitoring methods and enantioselective toxicity assays. In order to achieve an improved understanding of the wide-ranging implications of enantioisomerism of CDs on environmental risk assessment, the attention given to enantioselective approaches cannot be overlooked.

In a multi-residue enantioselective monitoring program performed in England, eighteen pairs of enantiomers and three single enantiomers of chiral pharmaceutically active compounds were assessed in wastewater from five different WWTPs and surface water from a large river catchment [8]. This study was monitored by LC–MS/MS with TQD and a chiral-AGP CSP was used for the enantioseparation. The stereoselective degradation of those chiral compounds using lab-scale microcosm (simulated activated sludge bioreactors) and river water microcosm was simultaneously evaluated in that study. Monitored samples showed both spatial and temporal variations and enantiomeric enrichment of the studied CDs, despite their racemate formulations commercialized [8]. The same enantioselective patterns were observed in effluents and receiving waters. CDs exhibited different enantioselective degradation under different dissipation experiments, namely trickling filters, sequencing batch reactors, and activated sludge [8]. Nevertheless, naproxen stereoselective degradation was reported to be highly variable among different WWTPs

Table 2
Summary of the different enantioseparation methods used for biodegradation studies.

Compound	Matrix	Sample preparation	Analytical methodology	Chromatographic conditions	LOD	LOQ	Reference
Climbazole and climbazole-alcohol (metabolite)	Wastewater and sludge	SPE (Oasis HLB, 6 mL, 500 mg, and Oasis MCX, 6 mL, 200 mg); PLE	LC–HRMS	Lux amylose-2 chiral column (150 × 2 mm i.d., 3 μm), acetonitrile/ultrapure water (65:35, v/v)	–	0.2–0.8 ng L^{-1} (constructed wetland matrices); 1.8 –2.6 ng g^{-1} (sludge)	[56]
Aminorex, CIBP, cephalexin, DNAP, florfenicol, HIBP, IBP, ifosfamide, IDP, KET, mandelic acid, 2-phenylpropionic acid, PZN, tetramisole hydrochloride, levamisole, cephalexin, 3-N-dechloroethylifosfamide, dihydroketoprofen, NAP, 10,11-dihydro-10-hydroxycarbamazepine, fexofenadine hydrochloride, CTZ dihydrochloride, and CHP	Activated sludge and river water (microcosm bioreactors)	SPE (HLB-MAX in tandem)	LC–MS/MS	Chiral-AGP column (100 × 2 mm i.d., 5 μm), 10 mM ammonium acetate with 1% acetonitrile (pH 6.7)	–	–	[8]
FLX, VFX, ODMVFLX, CIT, DCIT, MTZ, ATE, MET, STL, PHO, ALP, SBT, AMP, MAMP, MDMA and MDA	Wastewater	SPE (HLB)	LC–MS/MS	Chiral-CBH column (100 × 2 mm i.d., 5 μm), water/1 mM ammonium acetate in 2-propanol (90:10, v/v); Chirobiotic V (vancomycin) column (250 × 2.1 mm i.d., 5 μm), 0.005% formic acid in methanol/4 mM ammonium acetate aqueous solution	0.03 –28.74 ng L^{-1}	0.11 –95.81 ng L^{-1}	[57]
OFX and LVX	Mineral salts medium and activated sludge	Centrifugation	LC–FD and LC –MS/MS	Chirobiotic R (ristocetin A) column (150 × 2.1 mm i.d., 5 μm), 0.45% triethylamine in aqueous solution (pH 3.6 adjusted with acetic acid)/ethanol (80/20, v/v)	2.5 μg L^{-1}	5 μg L^{-1}	[6,27]
EP and PEP	River simulating microcosms	SPE (HLB)	LC–MS/MS	Chiral-CBH column (100 × 2 mm i.d., 5 μm), water/1 mM ammonium acetate in 2-propanol (pH 5.0) (90/10, v/v)	–	5.6 ng L^{-1}	[58]
Dufulin	Corn plants and corn	Solid–liquid extraction	LC–MS/MS	Chiralpak IC column (250 × 4.6 mm i.d., 5 μm), acetonitrile/water (45/55, v/v).	0.01 –0.04 mg kg^{-1}	0.01 –0.02 mg kg^{-1}	[59]
HCH, chlordane, *o,p′* DDE, *o,p′* DDD and *o,p′* DDT	Biota extracts from marine species	Freeze-drying and soxhlet-extraction	GC–ECD	HP-5 and DB-1701 columns (30 m × 0.25 mm i.d., 0.25 μm film thickness)	0.01 –0.05 ng g^{-1}	–	[60]

ALP: alprenolol; AMP: amphetamine; ATE: atenolol; CIBP: carboxyibuprofen; CIT: citalopram; CHP: chloramphenicol; CTZ: cetirizine; DCIT: desmethylcitalopram; DDD: dichlorodiphenyldichloroethane; DDE: dichlorodiphenyldichloroethylene; DDT: dichlorodiphenyltrichloroethane; DNAP: desmethylnaproxen; EP: Ephedrine; FLX: fluoxetine; GC–ECD: gas chromatography–electron capture detector; HCH: hexachlorocyclohexane; HIBP: hydroxyibuprofen; HLB: hydrophilic–lipophilic balance; HPLC–FD: high performance liquid chromatography–fluorescence detector; IBP: ibuprofen; i.d.: internal diameter; IDP: indoprofen; KET: ketoprofen; LC–HRMS: liquid chromatography–high resolution mass spectrometry; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; LVX: levofloxacine; MAMP: methamphetamine; MAX: mixed-mode anion-exchange; MCX: mixed-mode cation exchange; MDA: 3,4-methylenedioxyamphetamine; MDMA: 3,4-methylenedioxymethamphetamine; MET: metoprolol; MTZ: mirtazapine; NAP: naproxen; ODMVLX: *O*-desmethylvenlafaxine; OFX: ofloxacine; PEP: pseudoephedrine; PLE: pressurized liquid extraction; PHO: propranolol; PZN: praziquantel; SBT: salbutamol; SPE: solid phase extraction; STL: sotalol; VFX: venlafaxine.

located in the same region and using similar treatments, which reinforce the variability of microbial communities and its influence on the degradation performance [8]. Furthermore, this changeability between WWTP performances is one of the major impairments when comparing removal efficiencies of emerging organic pollutants from different studies and the reason why many compounds are reported in the literature with so divergent removal rates.

Zhang et al. (2018) applied an enantioselective method using a Lux-cellulose-1 CSP and LC QTOF operating under reversed

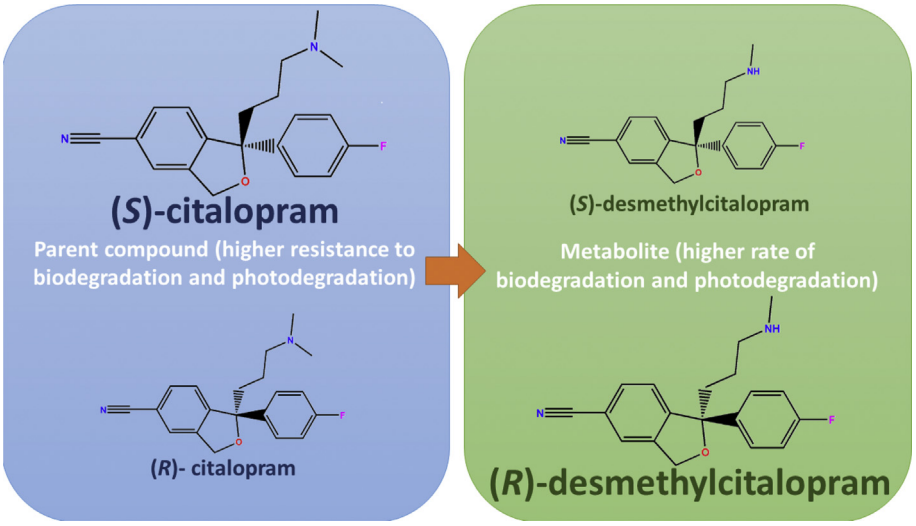

Fig. 3. Representative example of a parent CD (citalopram) that was found with an EF < 0.5 and which desmethylated metabolite (desmethylcitalopram) was determined with an EF > 0.5 in a degradation study [57] using spiked activated sludge microcosms under dark biotic conditions.

elution mode to determine the removal of prothioconazole and its main metabolite prothioconazole-desthio in soils from different origins, under native and sterile conditions [45]. In that study, racemization was not observed and each enantiomer of the parent chiral triazole was degraded into the corresponding form, i.e. *R*- and *S*-prothioconazole were transformed to *R*- and *S*-forms of the metabolite, respectively. *R*-prothioconazole was preferentially removed in the five types of soils and this conversion was ascribed to microbial degradation [45]. In turn, the metabolite had a lower half-life and no remarkable stereoselective degradation was verified, with EF variations being observed only in two soils.

Other emerging concern in this field is that enantiomers can show stereoselectivity in biotransformation pathways and therefore in the bioaccumulation processes up the food web, which may be proved by the relative accumulation of the enantiomers in biota and variations in EF. Recently, Zhou et al. [60] studied the chiral signatures of organochlorine pesticides (OCPs) in marine organisms of a food web from China, where most of the studied marine organisms contained non-racemic OCPs residues. In that study, the *in vivo* biotransformation of chiral OCPs was found to be minor and enantioselective bioaccumulation did not occur. Another study dealing with the determination of dufulin enantiomers in corn plants and corn by LC/MS/MS using a Chiralpak IC column [59], showed that both enantiomers of dufulin disappeared fast in the corn plant, and stereoselectivity was not observed during degradation.

2.3. Forensic purposes

Regarding enantiomeric analysis of drugs in environmental matrices for forensic investigations, a similar pattern to that of monitoring studies is evident, considering that the matrices are the same, as described in our recent review [19]. In fact, LC–MS/MS appears as the most common analytical methodology for the enantiomeric analysis of CDs of forensic interest in environmental matrices (Table 3). For example, wastewater analysis to estimate consumption patterns has been reported using LC–MS/MS with TQD and the CSP Chiralpak® CBH. In a study focused on the spatial and temporal variation in the enantiomeric profile of CDs in eight European cities, WBE and enantioselective analysis were combined to evaluate trends in illicit drug usage in the context of their consumption *vs* direct disposal, along with their synthetic production routes [62]. A seven-year study was reported describing the enantiomeric profile of amphetamine and methamphetamine in wastewater to evaluate the consumption of these substances in regional and urban areas of Queensland, Australia [63]. For that, a LC–MS/MS with a QTrap and a Chirobiotic V2 CSP were used for enantioseparation. Both the enantiomeric profile and the ratio amphetamine/methamphetamine gave information about consumption in the area and possible synthetic pathways. The (*R*)-(−)-methamphetamine enantiomer was also identified in various samples, suggesting that the synthesis process could be based on phenyl-2-propanone. This information is important for authorities and for understanding drug trafficking. Another WBE study was conducted for the first time in the African continent to investigate the consumption of illicit drugs [64]. In that study, the enantiomeric profile showed the occurrence of non-racemic 3,4-methylenedioxymethamphetamine, methamphetamine and benzoylecgonine and that methamphetamine was the most consumed illicit drug in that region.

Though LC–MS/MS is the most common analytical methodology for forensic environmental research, it is interesting to observe that GC–MS is still often used to analyze few classes of CDs, including amphetamine related substances and cathinone in biological and seized samples by indirect methods (Table 3) [65,66,69]. A recent example of a GC method allowing the multiresidue analysis in a single chromatographic method, described an indirect GC–MS method for the enantiomeric separation of amphetamine related compounds, antidepressants and β-blockers [14].

Two-dimensional GC methods have also been proposed (GCxGC) for forensic applications [70], with several advantages over single dimensional chromatography, namely the significant increased peak capacity (selectivity), increased sensitivity and reduced analysis time by eliminating injections on multiple chromatographic phases. Other analytical methodologies such as SFC and CE have been reported for the enantioseparation of CDs with forensic interest, but in biological samples [19,36,67]. A recent example of simultaneous chiral impurity analysis of methamphetamine and its precursors was reported using an amylose-based CSP and a SFC–MS/MS method that can provide information on methamphetamine manufacturing methods in clandestine laboratories [68].

Table 3
Summary of the different enantioseparation methods used for forensic purposes.

Compound	Matrix	Sample preparation	Analytical methodology	Chromatographic conditions	LOD	LOQ	Reference
4-EMC, 3-MMC, benzedrone, clephedrone, 5-MAPB, 5-EAPB, 6-EAPB, 5-APDB, 6-APDB, 5-APB, ethyphenidate, thiothinone, MTTA	Recreational drugs	Derivatization procedure with (*S*)-TPC	GC−MS	HP5-MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness); helium	–	–	[65]
AMP, MAMP, MDMA, norketamine, FLX, norfluoxetine, PHO, ALP, bisoprolol, MET, sertraline, paroxetine	Wastewater influent	SPE (MCX); Derivatization procedure with 0.5% *R*-(−)-MTPACl and MSTFA	GC−MS	Capillary column (30 m × 0.25 mm i.d. × 0.25 μm, cross-linked 5% diphenyl and 95% dimethyl polysiloxane)	0.03−26 ng L^{-1}	0.15 −104.2 ng L^{-1}	[14]
AMP, MAMP, MDMA, MDA, HMMA, mephedrone, EP, PEP, norEP	Wastewater influent (24-h composite)	SPE (HLB)	LC−MS (triple quadrupole)	Chiralpak® CBH HPLC column, (10 cm × 2.0 mm i.d., 5 μm) with a chiral-CBH guard column (10 × 2.0 mm, 5 μm); 1 mM ammonium acetate/methanol (85:15, v/v).	0.5−5.9 ng L^{-1}	0.6−29.5 ng L^{-1}	[62]
Cocaine, MDMA, HMMA, AMP, MAMP, mephedrone, EP, PEP, norEP	Composite raw wastewater	SPE (HLB)	LC−MS (triple quadrupole)	Chiralpak® CBH HPLC column (10 cm × 2.0 mm i.d., 5 μm) with a chiral-CBH guard column (10 × 2.0 mm, 5 μm); 1 mM ammonium acetate/methanol (85:15, v/v).	0.1−5.9 ng L^{-1}	0.3−29.5 ng L^{-1}	[64]
AMP, MAMP	Urine	Derivatization procedure with 0.1 M (*S*)-TPC	GC−MS	Narrow-bore capillary GC column DB-5 (10 m × 0.1 mm i.d., 0.4 μm film thickness)	–	–	[66]
AMP, MAMP	Wastewater influent (24-h composite)	SPE (HLB)	LC−MS/MS (QTrap)	Chirobiotic V2 column (250 mm × 2.1 mm, 5 μm); methanol, acetic acid and ammonium hydroxide (100:0.1:0.025, v/v/v)	0.2 ng L^{-1} −1.0 ng L^{-1}	–	[63]
AMP	Urine	Semi-automatic sample extraction Tecan Freedom Evo pipetting robot; Evolute® Express CX 96-well plate (30 mg)	UHPSFC−MS/MS (triple quadrupole)	Chiralpak® AD-3 [amylose-tris-(3,5-dimethylphenylcarbamate] (150 mm × 2.1 mm, 3.0 μm); CO_2 and 0.2% cyclohexylamine in 2-propanol	10 ng mL^{-1}	25 ng mL^{-1}	[67]
MAMP, EP, PEP, norEP, norPEP, ClPEP, MeEP, DMA, and AMP	Seized material		UHPSFC:MS/MS (QTOF)	Chiralpak AD-3/SFC (150 mm × 3 mm i.d., 3 μm particle size); CO_2/co-solvent (propanol spiked with 0.3% cyclohexylamine)	0.2−1.3 ng	–	[68]
MAMP	Seized material	Derivatization procedure with trifluoroacetic anhydride	GC−FID	BETA DEX 225 capillary column (30 m × 0.25 mm i.d., 0.25 mm film thickness)	–	–	[69]

ALP: alprenolol; AMP: amphetamine; 5-APDB: 5-(2-aminopropyl)-2,3-dihydrobenzofuran; ClPEP: chloropseudoephedrine; DMA: dimethylamphetamine; 5-EAPB: 1-(benzofuran-5-yl)-Nethylpropan-2-amine; 6-EAPB: 1-(benzofuran-6-yl)-Nethylpropan-2-amine; 4-EMC: 4-ethylmethcathinone; EP: ephedrine; FID: flame ionization detector; FLX: fluoxetine; GC−MS: gas chromatography coupled to mass spectrometry; HLB: hydrophilic-lipophilic balance; HMMA: 4-hydroxy-3-methoxymethamphetamine; i.d.: internal diameter; LC−MS: liquid chromatography coupled to mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; MAMP: methamphetamine; MDMA: 3,4-methylenedioxymethamphetamine; 5-MAPB: 1-(benzofuran-5-yl)-N-methylpropan-2-amine; MeEP: methylephedrine; MS: mass spectrometry; 3-MMC: 3-Methylmethcathinone; MS/MS: tandem mass spectrometry; MET: metoprolol; MSTFA: *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide; MTPA-Cl: Methoxy-α-(trifluoromethyl)phenylacetyl chloride; MTTA: Mephtetramine; NorEP: norephedrine; norPEP: norpseudoephedrine; PEA: Phenethylamine; PEP: pseudoephedrine; PHO: propranolol; QTOF: quadrupole time of flight detector; SPE: solid phase extraction; MBDB: 3,4-methylene-dioxyphenyl-2-butanamine; MDA: 3,4-methylenedioxyamphetamine; (*S*)-TPC: (*S*)-(−)-trifluoroacetylprolyl chloride; UHPSFC−MS/MS: ultra-high performance supercritical fluid chromatography−tandem mass spectrometry.

3. Conclusions and future challenges

A better knowledge about the environmental occurrence of CDs, their fate, distribution, (bio)degradation, ecotoxicity and even the profiling for forensic purposes clearly depends on the results of appropriated analytical methodologies for quantification of the enantiomers. In this field, enantioselective LC−MS/MS methods with TQD analysers are currently the most used analytical methodologies due to the high versatility, sensitivity and practicability in routine setting. Though environmental impact of solvents

consumed should not be neglected and has been pointed out as one main LC drawback, the reduction of particle size and diameter of CSPs along with the replacement of hazardous solvents by greener alternatives can substantially reduce solvent consumption, waste production and environmental impact.

SFC is an emerging and greener analytical methodology over LC due to its low waste production and possible use of CSPs commonly used in LC systems, offering fast and efficient separations. Nevertheless, GC represents the second most used analytical method for CD analysis of volatile and semi-volatile compounds, offering high efficiency and an eco-friendly approach.

Considering detectors, TQD appears as the most used for quantification purposes. Though the use of exact mass for unequivocal confirmation of non-target compounds is crucial, only few studies reported this approach.

This paper highlights the importance of enantioselective method development for an accurate evaluation of CDs for the purposes herein reviewed. However, much needs yet to be done in the field of chiral environmental chemistry in order to get insights on the environmental behaviour, toxicity, risks and future regulation.

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